Citation:

Kalmbach RD, Choumenkovitch SF, Troen AM, D'Agostino R, Jacques PF, Selhub J. Circulating folic acid in plasma: Relation to folic acid fortification. *Am J Clin Nutr.* 2008; 88 (3): 763-768.

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Study Design:

Cross-sectional study (data from a longitudinal study)

Class:

D - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To assess the effect of folic acid fortification implementation on circulation concentration of folic acid (FA) and 5-methyltetrahydrofolate (5MeTHF) in the Framingham Offspring Cohort.

Inclusion Criteria:

- The offspring of the original Framingham cohort
- The spouse of the offspring.

Exclusion Criteria:

- Individuals examined between October 1996 and August 1997 were excluded, because the study concentrated on fortification throughout this period and the subjects' folic acid intake was uncertain
- If 12 or more food items were left blank in the food frequency questionnaire (FFQ) or if reported energy intakes were less than 600 kcal or more than 4,000kcal by the FFQ, the related dietary data were excluded from analysis.

Description of Study Protocol:

Recruitment

- The Framingham Heart Study was set up in Framingham, MA, in 1948. Its original cohort included 1,644 husband-wife pairs and 378 persons whose parents had cardiovascular disease (CVD)
- The Framingham Offspring Study in 1971, enrolled the offspring of those subjects and the spouses of the offspring.

Design

The current paper was based on cross-sectional data from a longitudinal study.

Dietary Intake/Dietary Assessment Methodology

- Dietary intake was assessed by a semi-quantitative FFQ
- Subjects were asked about frequency of food consumption in the past year, vitamin and mineral supplements and type of breakfast cereal most commonly consumed.

Blinding Used

Folic acid and 5MeTHF concentrations were measured with the use of the affinity-HPLC method with electrochemical(coulometric) detection method.

Intervention

Folic acid fortification policy.

Statistical Analysis

- Analyses were calculated separately for users of B vitamin supplements and for users of non-B vitamin supplements
- Total folate and 5MeTHF concentrations were log transformed before analysis and reported as geometric means with 95% CI, due to positive skewness
- Folic acid intake values: A square root transformation was used to normalize values due to its positive skewness
- Circulating folic acid concentrations: Median concentrations and prevalence of detectable and high concentrations were reported since they were skewed toward low values, and not all samples had detectable concentrations
- Detectable was defined as more than 0.18nmol per L as the limit of detection for the method and high was defined as 1.35nmol per L or more, the 85th percentile for the cohort at the sixth examination cycle
- The Kruskal-Wallis test was used to compared median values in subjects both exposed and unexposed to fortification
- SAS PROC GLM was used to assess the differences in prevalence of detectable and high-circulating folic acid with
- adjustment for age and sex with the use of the Tukey's adjustment for multiple comparisons
- SAS PROC GLM was used to compare 5MeTHF and total plasma folate geometric means in subjects exposed and unexposed to folic acid fortification

- Adjusting for folate intake with the use of the food composition database not modified to reflect folic acid fortification to ensure values were related to fortification and not to differences in dietary patterns of folate- or folic acid-containing foods
- Age- and sex-adjusted logistic regression analysis was used to calculate odds ratios for high circulating folic acid
- Relations between dietary vitamin intakes was adjusted for total energy intake
- A test for a trendwas performed by entering independent variables as continuous variables in the logistic regression model
- SAS PROC LOESS plotted predicted circulating folic acid concentrations and 95% CI by folic acid intake with a smoothing value of 0.3. It was used to describe graphically the relation between circulating folic acid and folate intake
- The statistical analyses was performed with SAS (version 9.1).

Data Collection Summary:

Timing of Measurements

The Offspring cohort received their sixth study examination, which was used for analysis in the current paper between January 1995 and August 1998.

Dependent Variables

- Folic acid and 5MeTHF concentrations:
  - Blood samples were taken from subjects after fasting for 10 or more hours
  - They were measured by the affinity-HPLC method with electrochemical (coulometric) detection method with serial modifications to increase throughput
- Folate activity:
  - Assessed with the use of an ESA Four-Channel CoulArray Detector (Bioscience Inc, Chelmsford, MA)
  - Quantification and identification of folates were compared to external and internal folate standards of known concentration
  - The CV for folate detection by the above method ranged between 5.2% and 8.6% (intra-assay) and 3.2% and 7.3% (inter-assay)
  - The limit of quantification for folic acid was 0.19nmol per L, and the limit of detection was 0.18nmol per L
- For these analyses, the limit of detectable folic acid (0.18nmol per L) was used to describe detectable folic acid.

Independent Variables

- Dietary intake:
  - Assessed by FFQ
  - To estimate intake of specific nutrients, the frequency of consumption was multiplied by the nutrient content of the specified portions
- Folate intake from the FFQ was modified to reflect fortification. That is, foods enriched with folic acid after implementation of mandatory fortification were recalculated with the use of data from a study that measured the folate content of foods after fortification. The foods recalculated included bread, corn grits, rice, pasta, corn meal, muffins, pancakes, crackers,

pizza, cookies, brownies, doughnuts, cakes, sweet rolls and pie. Ready-to-eat cereals that were fortified before mandatory fortification were modified if the measured folic acid content exceeded published database values. For all enriched cereal grain products, the original total folate values used in the FFQ database were replaced by the more recently measured total folate values, and a new variable representing measured folic acid intake was created
- Folic acid intake from supplements was assessed with the use of data from the FFQ, which asked information on supplement brand, type and frequency of consumption. Analyses were separate for non-supplement and supplement users
- Total folate intake as micrograms (mcg) of dietary folate equivalents (DFEs) were calculated by adding the amount of folate consumed as natural folate (in mcg) to the amount of folate consumed as folic acid (in mcg) multiplied by 1.7 to account for the increased bioavailability
- The implementation of folic acid fortification of flour and cereal grain by January 1998.

Control Variables

- Age, sex, total folate intake (total folate does not include FA added from fortification of grain products, other than ready-to-eat breakfast cereals) and total energy
- In data not shown, body mass index (BMI), smoking, alcohol intake, caffeine intake, natural folate intake, plasma concentrations and intake of vitamins B_6 and B_{12} were also adjusted in the model.

Description of Actual Data Sample:

- *Initial N:*
  - In 1971, the Framingham Offspring Study had 5,135 of the 6,838 eligible persons to participate in the first examination
  - There were 3,532 who participated in the sixth study examination between January 1995 and August 1998
- *Attrition (final N):* After application of exclusion criteria in the current study there were:
  - 705 non-supplement users
  - 398 supplement users not exposed to fortification
  - 355 non-supplement users
  - 245 supplement users exposed to fortification
- *Age:*
  - 29 to 86 years at the sixth examination
  - The average in each of the four groups described above was between 57 and 60 years
- *Location:* United States (The original Framingham Study was established in Framingham, MA).

Summary of Results:

TABLE 1: 5-Methyltetrahydrofolate (5MeTHF) and Circulating Folic Acid (FA) Status in Subjects from the Framingham Offspring Study Before and After Fortification, by B Vitamin Supplement Use[1]

	No B Vitamin Supplements	B Vitamin Supplements

	Before	After	Before	After
Subjects (N)	705	355	398	245
Male (%)	53.3	51.5	40.6	43.4
Age (year)[2]	57.4 (32 to 80)	59.9 (33 to 86)	58.0 (29 to 78)	59.6 (33 to 85)
FA intake (mcg per day)[3,4]	32.4 (27.9, 37.0)[a,5]	241.4 (224.1, 259.2)[b]	399.4 (378.2, 421.2)[c]	601.4 (568.6, 635.1)[d]
Total plasma folate (nmol per L)[4,6,7]	19.5 (18.8, 20.4)[a]	37.2 (35.3, 39.4)[b]	30.8 (29.2, 32.9)[c]	40.6 (37.8, 43.5)[b]
Total 5MeTHF (nmol per L)[4,6,7]	19.0 (18.3, 19.9)[a]	36.3 (34.2, 38.3)[b]	30.1 (28.5, 31.9)[c]	39.2 (36.5, 41.9)[b]
Circulating FA (nmol per L)[8]	0.25 (0 to 15.18)[a]	0.50 (0 to 24.11)[b]	0.54 (0 to 19.78)[b]	0.68 (0 to 33.94)[c]
Subjects with detectable FA (%)[4,6,7,9]	55.0 (51.1, 58.9)[a]	74.7 (69.5, 79.9)[b]	72.5 (66.9, 78.1)[b]	80.7 (74.2, 87.2)[b]
Subjects with high FA (%)[4,7,10]	9.4 (6.4, 12.4)[a]	19.1 (15.1, 23.1)[b,c]	15.9 (11.6, 20.2)[a,b]	24.3 (19.2, 29.3)[c]

1 To convert values for folate to nanograms per milliliter, divide by 2.266.
2 Mean; range in parentheses.
3 Folic acid intake square root transformed. Values were also adjusted for total energy.
4 Values in a row without common superscript letters are significantly different, P<0.05. Differences were adjusted for age and sex with the use ANCOVA with Tukey's post-hoc tests.
5 Mean, 95% CI in parentheses (all such values).
6 Values were log transformed; geometric mean reported.
7 Adjusted for age, sex and total folate intake (total folate does not include FA added from fortification of grain products, other than ready-to-eat breakfast cereals).
8 Median; range in parentheses. Values in a row without common superscript letters are significantly different as determined with the Kruskal-Wallis test.
9 Detectable FA was defined as 0.18nmol per L or more.
10 High FA was defined as 1.35nmol per L or more.

TABLE 2. Determinants of Circulating Folic Acid (FA) Concentrations at 85%[1] or Higher

Categories of Exposure	Subjects	OR (95%CI)	P
	N		
Dietary FA intake (mcg per day)[2]			
6.86 or less	265	1.0 (ref)	

6.86 to 70.18	263	0.79 (0.41, 1.54)	0.49
70.18 to 203.65	267	1.32 (0.74, 2.38)	0.35
More than 203.65	265	2.16 (1.22, 3.84)	0.007
P for trend[3]			<0.001
DFE intake (mcg per day)[2]			
276.94 or less	265	1.0 (ref)	
276.94 to 417.49	265	1.25 (0.65, 2.40)	0.50
417.49 to 630.23	265	1.67 (0.89, 3.16)	0.11
More than 630.23	265	2.84 (1.51, 5.35)	0.001
P for trend[3]			<0.001
B vitamin supplement use[4]			
No	1,060	1.0 (ref)	
Yes	643	2.28 (1.73, 3.01)	<0.001
Plasma folate (nmol per L)[4]			
10.74 or less	427	1.0 (ref)	
10.74 to 20.17	424	1.56 (0.94, 2.57)	0.08
20.17 to 33.72	425	2.52 (1.58, 4.03)	<0.001
More than 33.72	425	4.94 (3.16, 7.72)	<0.001
P for trend[3]			<0.001

1 Ref, referent; DFE, dietary folate equivalent; calculated as {natural folate intake (in mcg) + [folic acid intake (in mcg) X 1.7]}.
2 Multivariate adjusted for age, sex and total energy with logistic regression analysis. Does not include data from subjects who consume B vitamin supplements.
3 Calculated by modeling exposures as continuous variables.
4 To convert values for folate to nanograms per milliliter, divide by 2.266.

Other Findings

- Men were less likely to use B vitamin supplements than were women
- The proportion of subjects who used supplements increased after fortification
- Predicted circulating concentrations of folic acid by folic acid intake for the entire population: A positive linear association was observed between folic acid intake and circulating folic acid concentrations (P<0.001)
- The proportion of subjects with high circulating folic acid status (85 or higher percentile for the entire cohort) according to folate
- Intake expressed as DFEs shows an increased prevalence of high-circulating folic acid with the increased total folate intake (P for trend <0.001)
- Subjects consuming more than 1,000mcg of folate as DFEs had a 77.3% higher prevalence of circulating folic acid of 85% or higher than did subjects consuming between 400 and

1,000mcg of folate. The increase seemed to be due to folic acid intake and not to natural folate intake.

Author Conclusion:

The implementation of folic acid fortification resulted in increased circulating folic acid.

Reviewer Comments:

- *The biochemical and physiologic consequences of increased circulating folic acid were unknown; there was a need to understand the effects of chronic exposure to circulating folic acid*
- *The study used a modified affinity-HPLC with electrochemical detection method to detect circulating folic acid*
- *Because of a lack of information on what concentration was normal or might pose risk, the author defined high as higher than the 85th percentile*
- *The author argued that circulating folic acid was almost certainly derived from synthetic folic acid from fortified foods and supplements.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes

2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	N/A
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	???
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	N/A
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	Yes
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	Yes
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	???
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes

5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	Yes
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	N/A
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	Yes
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	???
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	N/A
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes

7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes